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Research Protocol

Pathology Section

Utility of Serum Lipids and CEA Levels as Novel Markers in Comparison to Various Histopathological Parameters and TNM Staging in Colorectal Carcinoma: A Research Protocol

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ABSTRACT

Introduction: Colorectal Cancer (CRC) is the third most widespread type of cancer around the globe. Elevated Carcinoembryonic Antigen (CEA) levels, a blood protein, are often observed in CRC patients.

Need of the study: Among gastrointestinal cancers, CRC is the most frequent cause of death. This research aims to investigate the potential link between serum lipid profiles, the levels of CEA, and the risk of developing CRC. Early detection and staging of CRC are crucial for effective management and improved outcomes.

Aim: To evaluate the efficacy of serum lipids and CEA levels as novel markers compared to different Tumour Node Metastasis (TNM) staging and histopathological parameters in CRC.

Materials and Methods: A cross-sectional study will be conducted in the Department of Pathology at Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi, Wardha, Maharashtra, India from October 2024 to April 2026. Peripheral blood samples will be collected from CRC patients to determine serum lipid profiles and CEA levels. Resected colon specimens will be processed and graded using TNM staging with appropriate staining. Karl Pearson's test (data distributed normally) or Spearman's test (data distributed non normally) will be used to conduct a correlation between continuous variables. A p-value of less than 0.05 will be considered statistically significant.

Keywords: Carcinoembryonic antigen, Lipoproteins, Tumour node metastasis

INTRODUCTION

Lung cancer is the most common and deadliest cancer for both sexes, followed closely by breast, prostate, and CRC in terms of diagnosis, and colorectal, stomach, and liver cancer for mortality. Among males, lung cancer is the most frequent, followed by prostate cancer and CRC for diagnosis, and liver and stomach cancer for mortality. Among females, breast cancer is the most common, followed by colorectal and lung cancer for diagnosis, and vice versa for mortality [1]. This is a rapidly globalising phenomenon. Poor lifestyle choices, such as smoking, poor diet, and not exercising, are associated with the risk of developing CRC [2,3].

The age-standardised incidence rate for CRC was 2.1 per 100,000 individuals per year, whereas the age-standardised death rate was 0.83 per 100,000 individuals annually [4]. If, the situation is not controlled by 2030, there are predicted to be about 2.2 million new cases and 1.1 million fatalities [5]. Epidemiological data shows that the majority of CRC patients are over 50 years old [6]. Total 10% of new cases worldwide are CRC instances, solidifying its position as the second most prevalent major carcinoma among females and the third most widespread major cancer among males globally [7].

Since, around 2010, cancer rates have been increasing in people under 65 years old. The incidence of regional-stage disease has risen by about 2%-3% annually, and for distant-stage disease, by 0.5%-3% annually, reversing the earlier trend of diagnosis from 1995 to 2005 [8]. A quick diagnosis is vital for prevention since, it enables detection and excision of polyps before they develop into cancer. Early-stage CRC rarely exhibits symptoms, contributing to a poor early detection rate [9].

The specific processes behind the development of CRC are difficult to identify due to its complicated pathophysiology. This uncertainty drives a constant search for novel strategies in studies of CRC. Liver metastases typically have a poor prognosis and a low overall survival rate. Hepatocellular Carcinoma (HCC) is the main type of primary liver cancer, followed by intrahepatic cholangiocarcinoma (CCA) [5]. Liver metastases occur more frequently than primary liver tumours [5]. Liver metastases can originate from various cancers, such as carcinomas, melanomas, lymphomas, sarcomas, and germ cell tumours. Carcinomas are the most common, making up 92%, with adenocarcinoma as the leading subtype at 75%. Liver metastases primarily come from colorectal carcinomas, with pancreatic, breast, lung, and gastric carcinomas following [10].

There exists a relationship between an elevated number of platelets (PLT) and CRC metastases [11,12]. CEA is an established indicator of CRC. It has become the most affordable test for early diagnosis of possibly resectable CRC and is frequently employed to monitor CRC relapse in individuals without evident symptoms. Research has shown a negative relationship between High-density Lipoprotein (HDL) levels and CRC risk [13,14]. In addition, there is a connection between dyslipidaemia-a group of diseases that includes elevated triglycerides and elevated Low-density Lipoprotein (LDL)-and a higher chance of developing CRC [15]. On the other hand, Lipoprotein (a), sometimes referred to as atherogenic lipoprotein (a), appears to provide a preventive measure against the onset of CRC [16].

Along with early diagnosis, accurate staging of CRC is pivotal in treating and managing the disease. Various CRC grading systems have been developed [17]; however, not one of the grading criteria is universally acknowledged and consistently utilised. Most classification systems assign tumours to three or four categories based on grade: G1 (well-differentiated), G2 (moderately differentiated), G3 (poorly differentiated), and G4 (undifferentiated). Histologic grade, despite

observer variability, is consistently predictive in multivariate analysis independent of stage. The high-grade of the tumour is an especially poor predictor [18].

Different biomarkers have been suggested for numerous cancers, of which CRC is only an example. Establishing highly predictive indicators for the early identification and staging of CRC is, therefore, important and has the potential to enhance the prevention and treatment of the disease. The present study aims to evaluate the efficacy of serum lipids and CEA levels as novel markers compared to different TNM staging in CRC and histopathological markers like desmoplasia, gland formation, necrosis, type of carcinoma.

Primary objective: To assess the efficacy of serum lipids and CEA levels as novel diagnostic markers for CRC.

Secondary objective: To compare the efficacy of this diagnostic method with histological parameters and to correlate them with TNM staging and histopathological parameters in CRC patients.

REVIEW OF LITERATURE

The originality of present research lies in its comprehensive approach to integrating serum lipid levels with CEA and histopathological parameters, aiming to develop a more robust diagnostic and prognostic tool for CRC. The present study targets clinicians and researchers focused on oncology and gastroenterology by addressing the gaps in current knowledge and proposing novel methodologies for CRC staging. The anticipated outcomes could significantly enhance CRC management, particularly in early detection and treatment stratification.

The diagnostic landscape for CRC has seen significant advancements through the exploration of various biomarkers and their potential prognostic and diagnostic utilities. In 2020, Li T et al., introduced a novel diagnostic approach focusing on serum lipids and cancer antigens [19]. The research involving 200 participants indicated that individuals with CRC exhibited elevated levels of Cancer Antigen 19-9 (CA19-9) and CEA, alongside decreased levels of HDL and Total Cholesterol (TC). These distinct biomarker alterations were notably observed in both post-surgeries. This combination proved to be a highly reliable diagnostic marker, underscoring the importance of a multi-marker approach.

Additionally, A. Attallah M et al., proposed a diagnostic score combining Mucin-1 (MUC1), Cytokeratin-1 (CK1), CEA, and CA19-9, which showed improved sensitivity and specificity for early-stage CRC diagnosis [20]. These innovative methods marked the beginning of a more accurate, non invasive, and comprehensive approach to improving CRC diagnostics and patient outcomes.

Ahn SB et al., explored the feasibility of using a proteomics blood test panel for early-stage CRC diagnosis. Utilising advanced techniques like SWATH-MS analysis, the study identified a subset of five protein biomarkers capable of distinguishing between early and late stages of CRC, showcasing the potential of proteomics in early diagnosis [21]. Furthermore, in 2020, Tümay V and Guner OS, demonstrated that normal levels of CEA and CA19-9 were associated with extended survival in CRC patients, advocating for their combined use in prognostic evaluations and follow-up surveillance [22].

These studies collectively underscored the significance of combining traditional tumour markers with innovative proteomic approaches to improve diagnostic precision and prognostic assessments in CRC. Jiang M et al., further contributed to advancements in diagnostics by examining the utility of multiple tumour markers and blood lipid indices [23]. Their retrospective study included 35 patients with colorectal adenoma, 64 with early-stage CRC, and 29 with advanced CRC. The findings indicated that integrating TC, HDL, CEA, and CA19-9 provided a robust diagnostic marker for CRC, enhancing the diagnostic process. Similarly, Pan Y et al., identified Apolipoprotein C1 (APOC1) as a promising biomarker for gastric cancer, highlighting its higher serum concentrations and significant correlations with clinical stages and lymph node metastases [24]. This discovery suggests the broader applicability of APOC1 in cancer diagnostics beyond CRC, demonstrating its potential both as a prognostic and diagnostic marker.

In 2023, Zhang X et al., examined the cholesterol level in HDL ratio (MHR) and the predictive value of monocytes to tumour markers, along with traditional markers such as CEA and CA19-9 (12). Their study, which involved 201 healthy participants and 202 CRC patients, revealed markedly higher levels of MHR, CEA, and CA19-9 in CRC patients than in healthy controls. The research underscored that elevated levels of these biomarkers serve as independent risk factors for CRC and that MHR correlates positively with tumour differentiation. The study highlighted the combined use of MHR, CEA, and CA19-9 in enhancing the predictive accuracy for CRC, as shown by the Area Under the Curve (AUC) of the ROC curve, emphasising the clinical utility of these combined markers in CRC detection [12].

The above studies illustrate the evolving and multifaceted nature of CRC diagnostics, highlighting the integration of traditional tumour markers, blood lipid indices, and advanced proteomic technologies to enhance early detection, prognostic evaluations, and patient management. The present study aims to contribute to the advancements in CRC diagnostics by exploring the relationship between various histopathological features, TNM staging, and the tumour markers serum lipid and CEA.

MATERIALS AND METHODS

A cross-sectional study will be conducted on patients diagnosed with colorectal carcinoma from October 2024 to April 2026, at the Department of Pathology, in collaboration with the Department of General Surgery and the Department of Biochemistry at Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), India. The Institutional Ethics Committee of Datta Meghe Institute of Higher Education and Research has approved the study under the reference number DMIHER (DU)/IEC/2024/74. Informed consent will be obtained from all subjects before conducting the study. The study has also been registered in the Clinical Trials Registry of India (CTRI) with the registration number CTRI/2024/07/069979, dated July 4, 2024.

Inclusion criteria: All patients presenting to the Surgery Department diagnosed with colorectal carcinoma arising de novo, confirmed by Histopathological Examination (HPE), will be included in the study. Patients with primary colorectal carcinoma who have not previously received treatment for this condition will also be included.

Exclusion criteria: Patients with a history of other malignancies, those who have received treatments for CRC, taking lipid-lowering medications, or with a history of any surgical procedure for the colon will not be considered for the study.

Sample size calculation: The study's estimated sample size was calculated using the formula [25].

$N=Z^2_{1-a/2} \times p(1-p)/d^2$.

Where,

$Z_{1-a/2}=1.96$ (Significance level=5%)

Prevalence (p) of CRC=87 per 100000 population=0.087% [26]

p=0.50

Estimated error (d)=5%=0.05

$N=(1.96)^2\times(0.00087)\times(1-0.00087)/(0.00087)^2=68$

Thus, the estimated sample size for present study is approximately 68 patients at a 5% level of significance. The study will include around 68 resected specimens from confirmed and planned colectomy cases received by the Department of General Pathology at JNMC.

Study Procedure

The patients will be randomly selected and categorised in four stages according to the TNM staging system from the American Joint Committee on Cancer: stage I, stage II, stage III, and stage IV [27]. For newly diagnosed patients, appropriate physical examination and clinical history will be obtained. For previously diagnosed cases, the clinical history will be obtained while keeping the inclusion and exclusion criteria in account.

Peripheral blood samples (5 mL) will be collected via venipuncture from patients at various stages during pre and post-admission, before the CRC surgery involving a complete mesocolic excision, and one month after surgery during follow-up. Blood samples will also be taken from healthy controls for comparison. In addition to the current information, the collection of the fasting venous blood samples CEA will be followed. Each blood sample will be measured at 5 mL and then processed to separate the serum through centrifugation at 3000 rpm for 15 minutes at 4°C. Subsequently, the isolated serum will be stored at -80°C to preserve for future analysis. Serum lipid and serum CEA levels will be estimated at the Department of Pathology and the Department of Biochemistry.

Grossing and TNM staging, according to the American Joint Committee on Cancer, will be used to assess tumour staging [27]. Routine parameters like serum cholesterol, LDL, and HDL will be assessed, along with serum CEA levels. These biological reference ranges will be considered while interpreting the biochemical parameters: Total Cholesterol (TC) (Desirable- 200 mg/dL, Borderline- 200-239 mg/dL, Enzymatic CHE/CHOD/POD Method), Triglycerides (Normal <150 mg/dL, Borderline- 150-199 mg/dL, Enzymatic (lipase/GK/GPO/POD) without correction of free glycerol), and HDL (Low- <40.0 mg/dL, High- >60 mg/dL), LDL (100-159 mg/dL), and Very Low Density Lipid (VLDL) (0-40 mg/dL), estimated by the Phosphotungustic acid method [26].

The TNM staging of tumours is a crucial prognostic factor that guides subsequent management decisions. The process begins with receiving the unopened specimen in formalin, bearing clinical description, verifying specimen identification, and noting the type of surgery to be performed. The entire specimen's length will be measured, and the tumour will be palpated from the outer aspect. Total Mesorectal Excision (TME) quality will be assessed before inking or opening the Abdominoperineal Resection (APR) and Anterior Resection (AR) specimens, and photographs will be taken for records.

The presence of tumour site perforation will be checked before inking the non personalised surface, avoiding the serosa. Upon inking, the specimen will be opened from the anterior side, beginning at either end of the tumour and extending 1 cm above and below it, while recording the distances of the resection margins. The tumour's location relative to the AR and APR in the rectosigmoid will be recorded, and the specimen will be fixed in formalin for 48 hours. After fixation, longitudinal mucosal resection margins will be sampled, the tumour's size will be documented, and the regions of interest will be submitted for microscopy. Every lymph node will be dissected, and the remaining bowel segment will be examined for abnormalities. Finally, mesorectum or peri-colonic fat will be sampled.

The specimen would then be submitted based on the tumour stage, including longitudinal mucosal resection margins and adjacent mucosa, and other glossy abnormal regions will be selected for further investigation. The tissue will be fixed in 10% formalin and subjected to paraffin embedding. These blocks of tumour embedded in paraffin from resected colectomy specimens will undergo automated tissue processing (manufacturer and info of instrument used) following grossing.

Four or five sections of the tumour, including the serosa and/or Circumferential Resection Margin (CRM), will be collected. The tumour sections (thickness) will be mounted on glass slides (Blue Star®) and subjected to haematoxylin (manufacturer) and eosin (manufacturer) staining.

Primary outcome: The TC, HDL, LDL, triglycerides, VLDL, and CEA levels will be evaluated as novel diagnostic markers for CRC. The relationship between these markers and the presence of CRC will be explored, determining their sensitivity and specificity in diagnosing CRC at various stages.

Sensitivity and specificity will be determined by comparing the test results of the diagnostic markers (e.g., serum lipids and CEA levels) against the confirmed diagnosis of CRC based on Histopathological Examination (HPE) and TNM staging. Sensitivity will be calculated as the proportion of true positive CRC cases (those correctly identified by the diagnostic markers) out of the total number of actual CRC cases.

Sensitivity=True positives/(True positives+False negatives)

Specificity will be calculated as the proportion of true negative cases (those correctly identified as not having CRC) out of the total number of non CRC cases.

Specificity=True negatives/(True negatives+False positives)

Secondary outcome: Evaluation of serum lipid profiles and CEA levels in correlation with histopathological parameters (desmoplasia, gland formation, necrosis, type of carcinoma) and TNM staging in CRC patients and to compare the diagnostic efficacy of these biomarkers against traditional methods to enhance staging precision. Additionally, the study will assess the effectiveness of these biomarkers in early versus late-stage CRC detection and their potential integration into routine clinical practice for improved management and early detection.

STATISTICAL ANALYSIS

Statistical analysis will utilise Statistical Packages for Social Sciences (SPSS) version 27.0. Continuous variables will be reported as mean±SD, median, and Interquartile Range (IQR), while categorical variables will be presented as frequency (%). The Kolmogorov-Smirnov test will be used to determine the normality of the data. The Wilcoxon rank-sum test or the Student's t-test (for normally distributed data) will be used to compare continuous variables between CRC patients and healthy controls (for not normally distributed data). More than three groups will be compared using one-way Analysis of Variance (ANOVA) (for normally distributed data) or the Kruskal-Wallis test (for not normally distributed data). Pearson's test (data distributed normally) or Spearman's test (data distributed non normally) will be used to conduct correlation between continuous variables. This analysis uses a Chi-square test to examine and assess the relationship and dependencies between different categorical variables. A value of p (less than 0.05) will be considered significant.

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